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THE INDUCTION OF PARTHENOCARPY IN PETUNIA[1]

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Numerous attempts to induce haploidy in plants have been made in the past, and the techniques have varied widely. These have included, among other methods, hybridization, both intergeneric and interspecific (Clausen and Mann, 1924; Gaines and Aase, 1926); cold and heat treatments (Blakeslee et al, 1922; Belling and Blakeslee, 1927; Randolph, 1932); injury to plant parts (Davis, 1931; Ivanov, 1938); irradiation of pollen with x-rays (Katayama, 1934; Ivanov, 1938; Rick, 1943); application of various sorts of pollen (Belling and Blakeslee, 1927; Jörgensen, 1928); and chemical treatments (Gustafson, 1936, 1942; van Overbeek et al, 1941). This report is concerned with three of these methods as they affect fruit development: the application of different pollen types and chemical and x-ray treatments. The plants from seed produced in the x-ray experiments will be dealt with in a later report.

PARTHENOCARPY INDUCED BY VARIOUS POLLENS

The effects of pollen extracts have been of some interest since the work of Fitting (1909) and Laibach (Laibach, 1933; Thimann, 1934). Redinger (1938) reported the production of homozygous diploids in *Petunia* through the application of pollen of closely related solanaceous forms. It was decided for this study to apply some pollen from plants bearing no close relationship to *Petunia* as well as some from closely related genera. Table I gives the results obtained.

Materials and Methods.—*Petunia* flowers were emasculated and pollinated with "foreign" pollen. Except where orchid pollinia were used, contamination was prevented by placing a piece of soda straw closed at one end with Scotch tape over the stigma and style. This could not be done with the pollinia for danger of dislodging them. All pollinia from a single orchid bloom were used in each

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treatment. The ovaries were allowed to remain on the plant until dried. After harvest, the thickness, texture, and shape of the ovary walls and activation of the ovules were examined under the binocular microscope and compared to those of normal fruits. Measurements were made along the long and short axes in millimeters. Controls were not pollinated after emasculation but stigmas and styles were covered. Only those treatments which gave positive results are cited below.

Treatments with orchid pollen.—Since the experiments of Fitting and Laibach, orchid pollen has been credited with containing relatively large amounts of some substance or substances, or the precursors of such substances, which initiates development of the ovary.

TABLE I

Pollen sources	Petunia strains pollinated											
	1A	2	2A	3	4	6	LaPal*	BT	Son	Noc	Total	Results
	Number of times used											
Cattleya Mossiae plus stigmatic substance						1					1	—
Stigmatic substance of *Cattleya Mossiae*					4	1					5	—
Cattleya "Priscilla" plus stigmatic substance				3	4	6					13	1+
Cymbidium sp.				16	11		2				29	4+
Cymbidium plus stigmatic substance				7	7	7					21	6+
Delphinium sp.	1	4		1					1		7	—
Lilium longiflorum	2	4	1	8		1					16	—
Lilium tigrinum	8		9	11	8	8	1	8			53	—
Paeonia sp.		6									6	—
Philadelphus sp.	1		2	4	6	2	2	1		1	19	—
Lycopersicum esculentum	1	5	3		1		5				15	—
Nicotiana affinis	1	10	4	9	19	26	7	18			94	4+; 5PF
Nicotiana glutinosa				10							10	—
Nicotiana Tabacum				15							15	1+
Salpiglossis sp.				45							45	2+; 1PF
										Total	349	

*Symbols: LaPal = La Paloma.
BT = Better Times.
Son = Sonata.
Noc = Nocturne
PF = Parthenocarpic fruit.
+ = Some activation.
— = No activation.

Results.—No parthenocarpic fruits were produced. Eleven of the treated ovaries showed mild activation, ten in the form of thickness and texture changes in the upper one-third to one-half of the wall. There was no increase in size. Only in one case was there any activation of the ovules. This ovary exhibited a texture and thickening change in the upper half of the walls (pl. 11, fig. 1), while two ovules at the top of the column developed sufficiently to be classified as distorted empty seeds.

In general, the activation of *Petunia* ovaries with orchid pollen appears to be very slight. It seems possible to bring about such slight activation in the walls without affecting the ovules. In the case where the ovules were activated, the orchid pollinia were accompanied by stigmatic substance.

Treatments with pollen of Nicotiana affinis.—Of the four activated ovaries, one showed a partial hardening of the upper one-third of the ovary wall; three showed a hardening in the upper tip of the wall accompanied by a slight activation of a few ovules at the top of the column. The five parthenocarpic fruits were smaller than normal fruits (5 × 3½ mm., 4½ × 4, 4½ × 3, 3½ × 3, 3 × 3). All the walls exhibited the thickness, texture, and shape of normal fruits (pl. 11, fig. 2). They contained hollow seeds and split when ripe. Two of these fruits contained some ovules which had apparently undergone lesser degrees of stimulation and had developed in some cases to flat and distorted integumental structures (pl. 12, fig. 2).

Treatments with Nicotiana Tabacum.—One ovary showed a hardening and thickening in the upper third of the walls; the ovules were unchanged.

Treatments with Salpiglossis pollen.—Three ovaries gave positive results. In two of these the upper one-third of the capsule showed a hardening on the outside; the inner surface was not shiny as in a normal mature fruit. Neither was any larger than an unpollinated ovary allowed to dry on the plant (2½ × 1½ mm., 3 × 1½). The size of the third fruit (4½ × 3½ mm.) indicated greater activity. The upper three-fourths of the wall had hardened and thickened; the inner surface had become somewhat shiny but ovules showed no activation.

PARTHENOCARPY INDUCED WITH 2, 4-D

The effectiveness of 2, 4-D in the production of parthenocarpic fruits has been amply demonstrated (Avery, 1947). Of the chemical substances used in this study in attempts to stimulate development of the egg, 2, 4-D, although giving no results parthenogenetically, did produce some interesting results parthenocarpically. In an initial test, 2, 4-D at 2 p.p. 100 in lanolin was applied to the stigmatic surfaces of 21 emasculated flowers and in all cases gave positive results. For the most part, these fruits were perfectly normal in appearance, splitting at maturity to reveal an abundance of hollow seeds. A few of these seeds, when punctured with a needle, were seen to have a small amount of whitish material inside. The three largest fruits measured 7 mm. along the long axis and 4 mm. along the short; the remainder showed a gradual decrease in size to the smallest which was 3½ × 2½ mm. Only one of these fruits (6 × 4 mm.) did not contain at least a few empty seeds, but contained only ovules which had obviously undergone an activation where development of the integument had fallen short of the hollow-seed stage.

Because of the pronounced effect of 2, 4-D at such a high concentration, it seemed advisable to check it at lower levels and in different media; accordingly, tests were run using the substance in lanolin, water and talc at concentrations of

1 p.p. 100,000, 1 p.p. 10,000, 1 p.p. 1,000, 1 p.p. 100 and 2 p.p. 100 in each medium.

Materials and Methods.—The pure acid was ground and mixed in lanolin or talc to the desired concentration; when water was used as a medium the material was dissolved in a few cc. of acetone and then properly diluted with distilled water. The paste, powder, or liquid was then applied to the stigmatic surfaces of emasculated flowers; contamination by pollen was prevented by the straw method.

TABLE II

Strain No.	Size (mm.)	Result	Ovules	Change in ovary walls
			2, 4-D in lanolin 1 p.p. 1000	
6	2½ × 1½	—	—	—
6	3 × 2½	Small PF	Some activated	Th, Tex, S
6	3 × 2½	Small PF	1 Hol. S, remainder activated	Th, Tex, S
6	5 × 4	PF	Hol. S	Th, Tex, S
6	4 × 2	Small PF	Activated	Th, Tex, S*
6	3½ × 3	Small PF	Hol. S	Th, Tex, S
6	4½ × 3	Small PF	A few Hol. S (distorted)	Th, Tex, S (upper ¾* of length, papery below)
6	3½ × 2	Small PF	Some activity opposite active part of wall	Th, Tex, S (upper ¾* of length, papery below)
6	3½ × 1½	Small PF	As above	As above*
6	3 × 2	Small PF	As above	As above*
			2, 4-D in lanolin 1 p.p. 100	
6	3½ × 1½	—	—	—
3	4 × 2½	—	—	—
6	3 × 2	—	—	—
6	4 × 1½	—	—	—
6	4 × 3	Small PF	Some activity opposite active part of wall	Th, Tex, S upper ¾*, papery below
6	6 × 4	PF	Some Hol. S, remainder active	Th, Tex, S
6	7 × 5	PF	Abundant Hol. S	Th, Tex, S
6	9 × 5½	PF	Some small round Hol. S, remainder active	Th, Tex, S
6	10 × 5	PF	Abundant Hol. S	Th, Tex, S
6	8 × 5	PF	Abundant Hol. S	Th, Tex, S
6	8 × 4	PF	Abundant Hol. S	Th, Tex, S
			2, 4-D in lanolin 2 p.p. 100	
6	3½ × 1½	—	—	—
6	2½ × 1½	—	—	—
6	3½ × 1½	—	—	—
6	6 × 5	PF	Abundant Hol. S	Th, Tex, S
6	7 × 6	PF	Abundant Hol. S	Th, Tex, S
6	7 × 6	PF	Abundant Hol. S	Th, Tex, S
6	6½ × 6	PF	Activated	Th, Tex, S
3	4½ × 4	Small PF	Activated	Walls soft

Abbreviations: Th = thickness; Tex = texture; S = shape; Hol. S = hollow seed (integument only); PF = parthenocarpic fruit; * = ovules merely activated.

Analysis of the fruits was carried out as before. Controls were treated with lanolin, talc, water, and water and acetone. Most of the plants used were of strain No. 6 but a few flowers of strains Nos. 3 and 4 and La Paloma were treated.

Treatments with 2, 4-D in lanolin.—At concentrations of 1 p.p. 100,000 and 1 p.p. 10,000 there were no positive results. The largely positive effects of the higher concentrations are given in Table II.

Treatments with 2, 4-D in talc.—At concentrations of 1 p.p. 100,000 (eleven stigmas treated), 1 p.p. 10,000 (fourteen stigmas treated), and 1 p.p. 1,000 (ten stigmas treated), no activity was observed. Of the eleven flowers treated at 1 p.p. 100, three responded, while in the ten flowers of the "2 p.p. 100" class, two indicated positive results. Table III deals only with the five positive results obtained.

TABLE III

Strain No.	Size (mm.)	Result	Ovules	Change in ovary walls
	2, 4-D in talc, 1 p.p. 100			
4	6½ × 4	PF	A few activated at top of column	Th, Tex, S
6	4½ × 2	Small PF	Activated	Th, Tex
6	3 × 1½	+	Active at tip of column	Th, Tex, upper ⅓ papery below
	2, 4-D in talc, 2 p.p. 100			
6	5 × 2½	Small PF	Upper ⅓ of column with small distorted Hol. S	Upper ⅔ Th, Tex, S, papery below
6	4 × 2	Small PF	Upper ⅓ of column active	Upper ½ Th, Tex, papery below

Treatments with 2, 4-D in water.—At 1 p.p. 100,000, twelve treated flowers gave no response. Three of eleven flowers treated at 1 p.p. 10,000, two of twelve flowers treated at 1 p.p. 1,000, one of eight flowers treated at 1 p.p. 100, and four of eleven flowers treated at 2 p.p. 100 gave positive results which are summarized in Table IV.

Concentrations of 1 and 2 p.p. 100 in lanolin gave by far the best results of the 2, 4-D treatments, but it seems unnecessary to go beyond 1 p.p. 100 (pl. 11, fig. 3). The resulting fruits ripened on the plant and split longitudinally, as do normal fruits, upon drying. They contained hollow "seeds"; that is to say no endosperm or embryo was present. These seeds are composed of ovular tissue, the integument, which apparently has been stimulated; they are normal in appearance except that they are usually smaller than true seeds and are often somewhat lighter

in color although they may be of characteristic darkness. The pattern of the normal seed coat is always apparent (pl. 12, fig. 3). There was no injury to plant parts through the lanolin mixture.

The poor results obtained with talc mixtures can probably be accounted for by the lack of solubility; apparently where positive results were obtained the stigma was unusually moist. No injury was manifest through talc treatments. The aqueous treatments, on the other hand, produced injury in twelve of the nineteen treated flowers in the classes 1 and 2 p.p. 100. Injury ranged from a single sepal with necrotic spots to complete browning of sepals and pedicel. There can be no doubt that injury is important in reducing the incidence rate of parthenocarpy in these groups. In addition to injury, another difficulty in using water as a medium is that it is extremely difficult, if not impossible, to confine the mixture to the stigmatic surface.

TABLE IV

Strain No.	Size (mm.)	Result	Ovules	Change in ovary walls
			1 p.p. 10,000	
4	5 × 4	Small PF	Activated	Th, Tex*
LaPal	3 × 1½	+	Slight activity	Upper ½ Th, Tex
LaPal	3½ × 2	+	Activity doubtful	Upper ½ Th, Tex
			1 p.p. 1,000	
LaPal	7 × 4	PF	Activated	Th, Tex, S
6	5 × 2	Small PF	Slight activity	Walls soft but capsule splitting*
			1 p.p. 100	
6	6¼ × 4½	PF	Hol. S	Th, Tex, S
			2 p.p. 100	
4	4 × 3	Small PF	Strong activity	Th, Tex
4	7 × 5	PF	Strong activity at top of column	Th, Tex, S
4	4 × 3	Small PF	No activity	Th, Tex
6	7 × 5	PF	Abundant Hol. S	Th, Tex, S

*Ovules merely activated.

PARTHENOCARPY IN X-RAYED OVARIES

Materials and methods.—No. 6 plants were supported so that the flowers rested on a ring covered with Scotch tape. The flowers were strapped in place with Scotch tape on either side of the ovary, care being taken to center the ovary under the target. The technical factors were target distance 15 cm., filter ½ mm. of aluminum, 120 KV, 10 milliamps, H.V.L. = 1.6 mm. of aluminum. Ovaries were treated with 2400, 3000, 3600, 4200, 4800 and 5400 r; the number of fruits

harvested at maturity in each dosage class was 12, 7, 7, 7, 12 and 7, respectively. Untreated pollen from La Paloma flowers was used; contamination after pollination was prevented.

Results.—There was considerable variation in the effect of radiation on the ovule as far as seed development was concerned. In the "2400 r" class fruits contained filled seeds, partially filled seeds, empty but normal-appearing seeds, highly distorted empty seeds, and ovules showing only signs of initial development. Low levels of ovule activation are difficult to assess because there is no way as yet to determine whether an ovule is arrested in development because of radiation damage or whether it simply did not receive enough growth substance following pollination.

Only two fruits in the "3000 r" class contained some filled, partly filled, and round empty seeds. The remainder contained highly distorted ovular structures and ovules indicating little or no activation.

Three fruits of the "3600 r" class contained some filled, partially filled, and empty seeds. Some of these seeds were found to contain a soft, milky material. The remainder contained highly distorted empty seeds and activated or inactivated ovules (pl. 12, fig. 4).

The fruits of the remaining classes (4200, 4800, and 5400 r) contained only distorted empty seeds and ovules at various stages of activation.

Table V gives the results of a germination test conducted in constant illumination of 100 foot-candles supplied by fluorescent "daylight" bulbs and temperature of 25° C. Seeds were sterilized in 3 per cent hydrogen peroxide and germinated in Petri plates on filter-paper moistened with Vickery's solution. Counts were made eleven days after sowing. A germination test is hardly a suitable index of x-ray damage since seeds that germinate may give rise to seedlings that die somewhat later. Furthermore, this test cannot be regarded as definitive because of the small number of seeds per sample.

TABLE V

Dosage r	Seed number per sample	Sample wt. (mg.)	Full germination to 2 cotyledons	Laggards	Total
2400	100	7.29	7	8	15
3000	100	6.39	5	3	8
3600	75	4.22	2	3	5
Control	100	11.48	24	23	47

Conclusions.—Treatment with 2400 r is often fatal to egg and polar nuclei. Many fruits in this class contained a large number of empty as well as filled seeds, indicating that often the integument alone had proceeded to final development. The empty seeds are frequently quite normal in appearance and difficult to distinguish from filled seeds. The integument thus appears more resistant to treatment by x-rays than the internal tissues of the ovule.

The crumpled appearance of the distorted empty seeds which occur in all classes might be taken as an indication of radiation damage to the integument rather than evidence of collapse of the internal tissues of the ovule. Yet empty seeds with the same degree of distortion are found when irradiated pollen is placed on the stigmas of untreated flowers. In this case the integument has not been treated and the subsequent distortion must be due primarily to collapse of internal ovular structure. The integument may suffer injury but it is difficult to distinguish between damaged and collapsed integument.

The ovary wall and placental column are more resistant to x-radiation than the other tissues of the ovary. The walls develop the texture, thickness, and shape of normal fruits and split at maturity even under large doses (pl. 11, fig. 4).

EFFECTS INDUCED WITH IRRADIATED POLLEN

Materials and Methods.—Mature pollen from shattered anthers of La Paloma flowers was gathered and placed in No. 2 gelatin capsules prior to radiation. The technical factors involved were the same as for the irradiation of ovaries. The capsules were held in place on the ring with small strips of Scotch tape. Following treatment the pollen was placed on the stigmatic surfaces of No. 6 flowers. Protection against undesirable pollination was provided. There were in all fourteen radiation classes. Beginning with 13,200 r and increasing at increments of 600 r, the treatments were carried on until a dosage of 18,000 r was reached. They were resumed at 20,000 r, and the following doses were given: 22,200, 23,400, 24,600 and 25,800 r. Eight to ten fruits were analyzed in each class.

Results: Classes 13,200 r to 17,400 r.—The seed set was abundant. In the classes through 16,800 the completely filled seeds exceeded the partially filled and empty seeds although this excess appeared to decrease as the dosage rose. There was a steady increase also in the number of ovules giving rise to flat, cup-shaped and distorted structures, indicating damage to male nuclei. In class 17,400 the filled seeds were about equal to the partially filled and empty seeds.

Class 18,000 r.—Seed was generally abundant, with filled seed equalling partially filled to empty seed in about half the fruits. In the remainder, the partially filled to empty seeds exceeded the filled. An increase in the number of flat, cup-shaped, and distorted structures arising from ovules activated to a somewhat lesser degree was apparent.

Class 20,000 r.—Filled seed appeared to be about equal to partially filled and empty seed. Large numbers of distorted ovular structures and ovules that had been merely activated were observed, indicating that an increasing number of ovules was receiving badly damaged male nuclei or was simply undergoing a purely chemical activation.

Classes 22,200 to 25,800 r.—In these groups there were fewer filled seeds than partially filled and empty seeds. The number of distorted ovular structures is far greater than the number of recognizable "seeds," whether empty or filled, indicating that most of the male nuclei have undergone damage (pl. 12, fig. 5).

The difficulty in measuring precisely the amount of damage sustained by the pollen grain is apparent in the following comparison. In class 23,400 r, nine fruits were analyzed. The average number of recognizable "seeds" (filled, partially filled, or empty) was 29 per capsule. The ratio of filled seeds to partially filled or empty seeds at one extreme was 1 to 9, at the other 0 to 50. The average was 1 to 36. Normal fruits of this size might contain from 100 to 250 viable seeds. The large number of ovules that had undergone stimulation but had failed to develop would therefore indicate a high degree of damage.

In class 25,800 r ten fruits were analyzed. The average number of recognizable seeds per capsule was 57.4, higher than in class 23,400, although again the number of ovules falling short of complete development is high when compared with the number of seeds occurring in a normal fruit. The ratio of filled to empty or partially empty seeds was 1 to 4.6. Although this is an extreme case, it illustrates the difficulty in giving a true evaluation of damage. Variation in the number of seeds, damaged or otherwise, or in the number of activated ovules, might be due in part to the number of pollen grains employed. However, it is more likely due to the degree of damage suffered by the pollen grain depending upon where and how it is hit.

In general, it seems safe to say that with increasing dosage, fewer filled seeds are developed, that the number of partially filled and empty seeds increases, and that finally the number of ovules merely undergoing some degree of activation increases. It would appear from examination of large numbers of activated ovules and completely hollow "seeds" composed only of integument that x-radiation of pollen grains, severe enough to kill nuclei, often does not nullify the stimulating effect of the activating substances or their precursors within the grains. The growth or activating components of the grains retain some ability to stimulate the ovary wall as well as the integument so that sometimes these fruits are of much the same size as a normal fruit (pl. 11, fig. 5).

The following tables indicate that no completely parthenocarpic fruits have been derived from x-rayed mature pollen, since even in the higher dosage classes, seeds capable of germination developed. Rick (1943), treating *Petunia* anthers immediately prior to anthesis, found that dosages as high as 50,000 r (200 KV, 10 ma, filters of ¼ mm. copper and ¼ mm. aluminum, target distance 10 cm., Wappler clinical unit) permitted the production of viable seed.

Germination test No. 1 was carried on under greenhouse conditions, the counts being made three weeks after sowing the seeds on moist filter-paper, following sterilization with Sarasan. Germination test No. 2 was carried on under the conditions described on page 103.

TABLE VI

GERMINATION TEST NO. 1 (Samples 100 seeds each)

Dosage r	Full germination to 2 cotyledons	Laggards	Total
Control	48	15	63
13,200	35	5	40
13,800	36	8	44
14,400	38	7	45
15,000	26	12	38
15,600	32	10	42
16,200	12	13	25
16,800	19	5	24
17,400	14	11	25
18,000	13	5	18
20,000	22	8	30
22,200	0	1	1
23,400	0	0	0
24,600	0	0	0
25,800	10	7	17

GERMINATION TEST NO. 2

Dosage r	Seed number per sample	Sample wt. (mg.)	Full germination to 2 cotyledons	Laggards	Total
13,200	100	7.20	21	10	31
13,800	100	7.03	20	20	40
14,400	100	8.44	19	16	35
15,000	100	7.00	20	18	38
15,600	99	8.24	20	15	35
16,200	100	7.04	6	6	12
16,800	100	6.64	19	7	26
17,400	100	7.29	4	12	16
18,000	100	5.83	9	4	13
20,000	100	6.40	11	5	16
22,200	100	4.65	2	1	3
23,400	55	2.23	0	0	0
24,600	55	1.85	0	0	0
25,800	100	5.41	6	2	8

PARTHENOCARPY INDUCED WITH POLLEN FROM X-RAYED ANTHERS

Since pollen grains collected at anthesis require such high dosages for inactivation, anthers were taken from La Paloma flowers about to open. At this time anthers contain pollen but are plump and juicy.

Materials and Methods.—The anthers were placed in a No. 2 capsule, irradiated, and then allowed to ripen and shatter within the capsule. The pollen was then applied to No. 6 flowers, with soda straws being used to prevent any additional pollination. The x-ray doses ranged from 5400 r to 13,800 r at increments of 1200 r. Only the most turgid anthers from a single flower were treated in each class because occasionally one anther may be non-functional.

Results.—Classes 5400 and 6600 r were the only ones in which any filled seeds were found (pl. 12, fig. 6). Variation in the effectiveness of radiation was apparent in these two groups; only one fruit in the "5400 r" class contained any filled seed while in all three of the "6600 r" group a few were found. No filled seeds were found in the remaining classes except in the "12,600 r" class where one fruit contained one filled seed. In general, it may be said that as the dosage increased, the number of empty seeds increased until the bulk of the ovules was merely in some stage of activation, some remaining completely unstimulated.

In the "12,600 r" class the ovary walls did not develop completely but remained papery at the base. The upper portions showed characteristic texture, thickness, and shape. These fruits were also the smallest obtained in addition to containing the least activated ovules. In this experiment there appears to be a decrease in the size of the fruit with increasing dosage, indicating injury to the pollen growth substances or their precursors.

None of the four flowers treated with 13,800 r pollen developed. This is not surprising in view of the effects of increasing dosage on fruit development. The fact that the flowers treated with pollen receiving a dosage of 10,200 r failed to develop either indicates variability in response or else that other factors were involved (pl. 11, fig. 6).

DISCUSSION

Murneek (1951) has concluded that synthetic growth substances are not in themselves always responsible for fruit development but rather that they stimulate in some fashion a hormone or hormones already present in female tissue. This view is not too far removed from that taken by many investigators with regard to the activity of pollen; that is to say, that the activity of pollen, aside from furnishing nuclei in the formation of embryo and endosperm, is based on a substance which sets into motion a hormone system resulting in ovary enlargement.

There are ample references to the hormone content of pollen grains in the literature of plant growth substances, and Muir (1951) sums up the situation when he states that pollen of all sorts probably contains auxin, but that it may vary in amount and in condition; auxin may exist in a free or bound condition or as a precursor, and failure to detect it has been due to faulty techniques. Wittwer (1951) contends, as has van Overbeek et al. (1941), that in an actual pollination the number of grains involved is too small to furnish adequate hormone material for fruit production. Muir's (1947) experiments are of particular interest here because of the relationship between *Nicotiana* and *Petunia*. Pollen of *N. Tabacum* was found to contain only small amounts of free hormone with somewhat larger quantities in the bound condition. The unpollinated pistil indicated no free hormone, but considerable hormone in the bound state. A water extract of pollen was found to release much larger quantities of bound hormone in the free condition from dried ovary tissue. In a later report (1951) he estimated

that following fertilization the auxin content in the ovary is 100 times greater than the maximum amount obtained from extraction of pollen. It was 30 times greater in the style. It would appear, then, that there is something in pollen other than its native hormone complement which instigates the release of hormones in the ovary following pollination. After fertilization the ovules become a rich source of hormones as indicated by the experiments of Wittwer (1943), Britten (1950), and others.

The development of integument and ovary wall need not in certain cases be dependent upon the development of endosperm and embryo. Studies with 2, 4-D and other substances have resulted in the production of parthenocarpic fruits filled with empty seeds. The use of foreign pollen, as shown here, occasionally results in parthenocarpic fruits containing empty seeds, the emptiness apparently due to genetic differences between sperm and egg, while the seed coat and ovary wall are stimulated by the less specific activators within the grain. Furthermore, pollen grains treated with x-ray dosages sufficient to render their nuclei genetically inactive, can still stimulate integument and wall growth although it has not been determined histologically as yet that fertilization followed by collapse of the system within the integument has not occurred. This last point deserves amplification. A glance at the data concerning the fruits produced with irradiated dry pollen shows that none of these was completely parthenocarpic. Even in the highest dosage class a few filled seeds developed and there were others partially filled. Since fertilized ovules are known to be rich sources of hormone, it is easy to visualize a diffusion of hormone material from fertilized to adjacent unfertilized ovules with the subsequent expansion of integument and wall tissues. Britten (1950), studying maize, concluded that naturally parthenocarpic fruits resulted from the activity of auxin products emanating from seeds developing close by. The spatial arrangement of parthenocarpic and normal fruits on the ear coincided with vascular supply. In these *Petunia* fruits, it would seem possible, even when male nuclei had been damaged, for fertilization to occur, and, providing that collapse of the fertilized egg apparatus did not take place too soon, a diffusion of hormones could begin. In the cases of parthenocarpic fruits produced by irradiating ovaries or turgid anthers, this does not appear to be as important a consideration, since the appearance of the integument indicates a very early collapse of the nucellus and they are usually completely empty. Radiation damage to the male nuclei had apparently been severe enough to prevent fertilization.

Whether integument can develop to any extent without development of the ovary wall remains to be seen. Some treatments in this study with 2, 4-D in lanolin at 1 p.p. 1,000 and at various concentrations in talc (when moisture was present) have resulted in small parthenocarpic fruits in which the only activated integuments were located on parts of the placental column opposite wall tissue showing normal thickening and texture. Those ovules opposite less-developed portions of the ovary wall such as the bases of these small fruits, which usually

remain thin and papery, showed little if any activity. To activate integument separately, an activator not stimulating other tissues would be necessary, and whether the space required for enlargement would be available without growth of the wall seems doubtful.

Since ovary walls and ovules can, under certain conditions, act independently, then 2, 4-D, when applied to the stigma of *Petunia*, is usually an activator for both systems. X-rayed pollen and the pollen of *Nicotiana affinis* would appear to be in the same category.

If we assume that it is possible for all pollen types to have within them certain activating substances in common but that the pollens of genetically related groups exhibit fewer and lesser differences among themselves, then it is possible to account for parthenocarpy arising as it does here from a combination of solanaceous pollen and *Petunia* stigmas. It is then possible to account also for the exceedingly mild activation provoked by the orchid pollen in *Petunia* ovaries. Such an explanation would require that basically similar pollen grains produce, or do not produce, results depending upon the orientation of these substances in a genetically suitable background. In short, they must find the proper kind of stigma. That nuclei involved in fertilization have a much stricter limitation placed upon them has been amply illustrated in the failures of numerous attempts to obtain seeds from certain interspecific or intergeneric crosses.

The activating substance or substances in pollen seems to be independent of the nucleus, in a functional sense at least, at the time of pollination, since parthenocarpic fruits tend to be produced by irradiated pollen although pollen nuclei have been damaged by x-rays. The substance appears to be more stable in the presence of x-rays than the nucleus. This stability is not as great when turgid anthers are irradiated as when dry pollen is treated as indicated by fruit size, and it is possible that such resistance varies with moisture content (Lea, 1947). The nuclei of dry pollen too require higher lethal doses than those in the moist anther, but here the question is further complicated in that the nuclei of dry grains are further removed in time from completion of meiosis than the nuclei of less mature grains.

SUMMARY

1. Fourteen types of pollen were placed on the stigmas of 349 *Petunia* flowers. Five of these pollen types were solanaceous, two of them (*Nicotiana affinis* and *Salpiglossis* sp.) producing parthenocarpic fruits which were somewhat smaller than normal fruits.

2. Parthenocarpic fruits have been produced in *Petunia* with 2, 4-D, x-rayed pollen, and x-rayed ovaries.

3. The effects of these methods are discussed with regard to fruit development.

4. The lethal dose for egg and accessory cells appears to be from 2400 to 3000 r under conditions outlined above. Completely lethal doses for nuclei of

moist pollen (in the anther) were about 6600 r and for dry pollen undetermined, but over 25,800 r. The ovary wall, the integument, and the placental tissue, perhaps because of their relative dryness, showed no ill effects from treatments up to 5400 r and responded normally to activating substances of pollen. The activator substances of pollen grains require a higher lethal dose than nuclei in both dry and moist pollen, although in treating moist pollen (in the turgid anther) these lethal doses are lower.

BIBLIOGRAPHY

Avery, George S., Jr. (1947). Hormones and Horticulture. McGraw-Hill, New York.

Belling, J., and A. F. Blakeslee (1923). Reduction division in haploid, diploid, triploid and tetraploid Daturas. Proc. Nat. Acad. Sci. U. S. 9:106–111.

————, and A. F. Blakeslee (1927). The assortment of chromosomes in haploid Daturas. La Cellule 37:355–361.

Blakeslee, A. F., J. Belling, M. E. Farnham and A. D. Bergner (1922). A haploid mutant in the Jimson Weed, *Datura stramonium*. Science 55:646–647.

Britten, E. J. (1950). Natural and induced parthenocarpy in maize and its relation to hormone production by the developing seed. Am. Jour. Bot. 37:345–352.

Clausen, R. E., and Margaret C. Mann (1924). Inheritance in *Nicotiana Tabacum*. V. Occurrence of haploid plants in interspecific progenies. Proc. Nat. Acad. Sci. U. S. 10:121–124.

Davis, B. M. (1931). Some attempts to obtain haploids from *Oenothera Lamarckiana*. Am. Nat. 65:233–242.

Fitting, H. (1909). Die Beeinflussung der Orchideenbluten durch die Bestäubung und durch andere Umstände. Zeit. Bot. 1:1–86.

Gaines, E. F., and Hannah Aase (1926). A haploid wheat plant. Am. Jour. Bot. 13:373–385.

Gustafson, F. G. (1936). Inducement of fruit development by growth promoting chemicals. Proc. Nat. Acad. Sci. U. S. 22:628–636.

————, (1942). Parthenocarpy: natural and artificial. Bot. Rev. 8:599–654.

Ivanov, M. A. (1938). Experimental production of haploids in *Nicotiana rustica* L. Genetica 20:295–386.

Jörgensen, C. A. (1928). The experimental formation of heteroploid plants in the genus *Solanum*. Jour. Genet. 19:133–211.

Katayama, Yoshievo (1934). Haploid formation by x-rays in *Triticum monococcum*. Cytologia 5:235–237.

Laibach, F. (1933). Versuche mit Wuchsstoffepaste. Ber. Deutsch. Bot. Ges. 51:386–392.

Lea, D. E. (1947). Actions of Radiations on Living Cells. MacMillan, New York.

Muir, R. M. (1947). Relationship of growth hormones and fruit development. Proc. Nat. Acad. Sci. U. S. 33:303–312.

————, (1951). The growth hormone mechanism in fruit development. (In Plant Growth Substances, edited by Folke Skoog. Univ. Wis. Press). pp. 357–364.

Murneek, A. E. (1951) Growth regulating substances in relation to reproduction of some horticultural plants. (In Plant Growth Substances, edited by Folke Skoog. Univ. Wis. Press). pp. 329–345.

Randolph, L. F. (1932). Some effects of high temperature in polyploidy and other variations in maize. Proc. Nat. Acad. Sci. U. S. 18:222–229.

Redinger, Karl (1938). Über die Entstehung diploiden Embryonen aus unbefruchteten, mit gattungsfremden Pollen bestäubten Samenanlagen von *Petunia nyctaginiflora*. Biol. Zentralbl. 58:142–151.

Rick, Charles M. (1943). Cytogenetic consequences of x-ray treatment of pollen in *Petunia*. Bot. Gaz. 104:528–540.

Thimann, K. V. (1934). Studies on the growth hormone of plants. VI. The distribution of the growth substance in plant tissues. Jour. Gen. Physiol. 18:23–34.

van Overbeek, J., M. E. Conklin and A. F. Blakeslee (1941). Chemical stimulation of ovule development and its possible relation to parthenogenesis. Am. Jour. Bot. 28:647–656.

Wittwer, S. H. (1943). Growth hormone production during sexual reproduction of higher plants. Missouri Agr. Exp. Sta. Res. Bull. 371. 58 pp.

————, (1951). Growth substances in fruit setting. (In Plant Growth Substances, edited by Folke Skoog. Univ. Wis. Press). pp. 365–377.

Explanation of Plates

PLATE 11—FRUITS

Fig. 1. Control (left); *Cymbidium* male × *Petunia* female (note activation in upper half); normal *Petunia* fruit at right.

Fig. 2. Three parthenocarpic fruits from the cross *Nicotiana affinis* male × *Petunia* female. Normal *Petunia* fruit at right.

Fig. 3. Control; 2, 4-D in lanolin, 1 p.p. 1,000; 2, 4-D in lanolin 1 p.p. 100; normal.

Fig. 4. Six fruits from x-rayed ovaries treated with normal pollen. (2,400; 3,000; 3,600; 4,200; 4,800; 5,400 r). Normal fruit at right.

Fig. 5. Two fruits resulting from pollen treated with 25,800 r applied to normal flower. Normal fruit at right.

Fig. 6. Six fruits resulting from pollen treated before anthesis and applied to normal flowers. The largest fruit from each dosage class is shown here (5,400; 6,600; 7,800; 9,000; 11,400; 12,600 r). Normal fruit at right.

PLATE 12—SEEDS

Fig. 1. Normal seeds, × about 5.33.

Fig. 2. Hollow seeds of *Nicotiana affinis* male × *Petunia* female, × about 5.33.

Fig. 3. 2, 4-D in lanolin, 1 p.p. 100, × about 5.33. Some crushed seeds have been added to show the hollow condition.

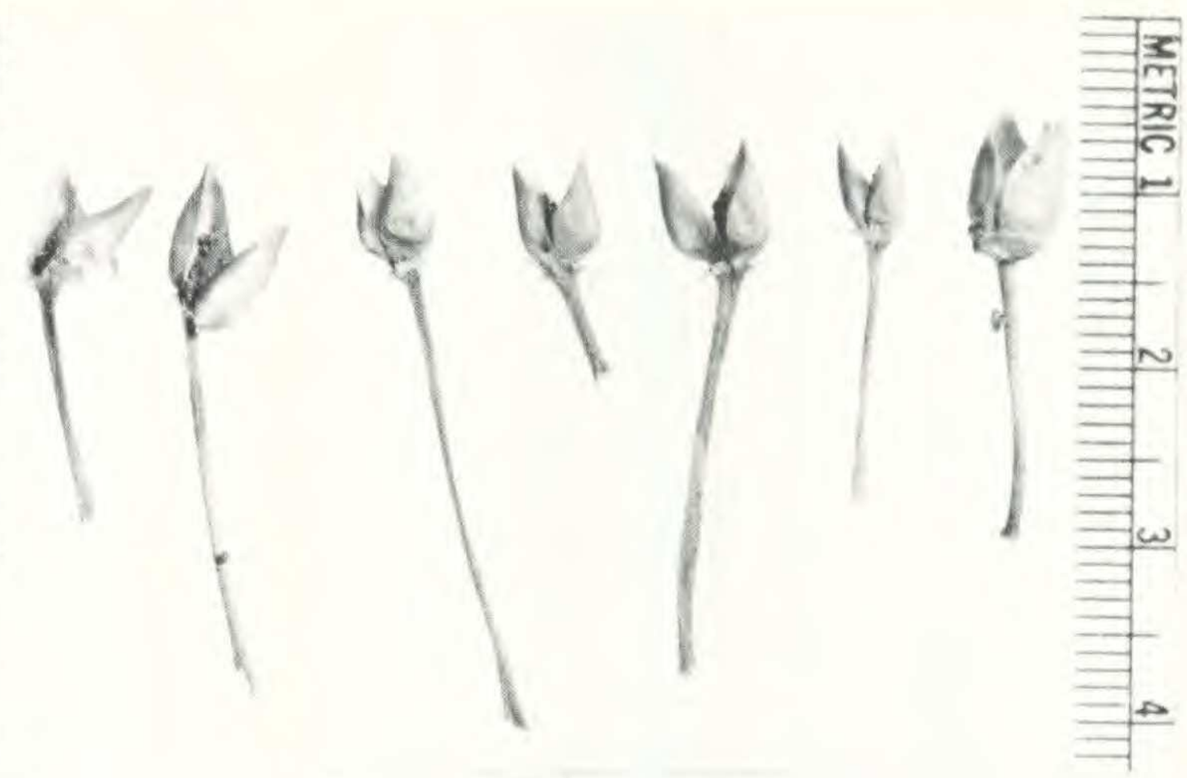
Fig. 4. Hollow seeds from ovaries treated with 3600 r × normal pollen, × about 5.33.

Fig. 5. Seeds from pollen treated with 25,800 r × normal flowers, × about 5.33. Some crushed seeds have been added to show the hollow condition.

Fig. 6. Seeds from normal ovaries × pollen from anthers irradiated at 5,400 r prior to anthesis, × about 5.33.


METRIC 1
2
3
4

1


METRIC 1
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METRIC 1
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METRIC 1
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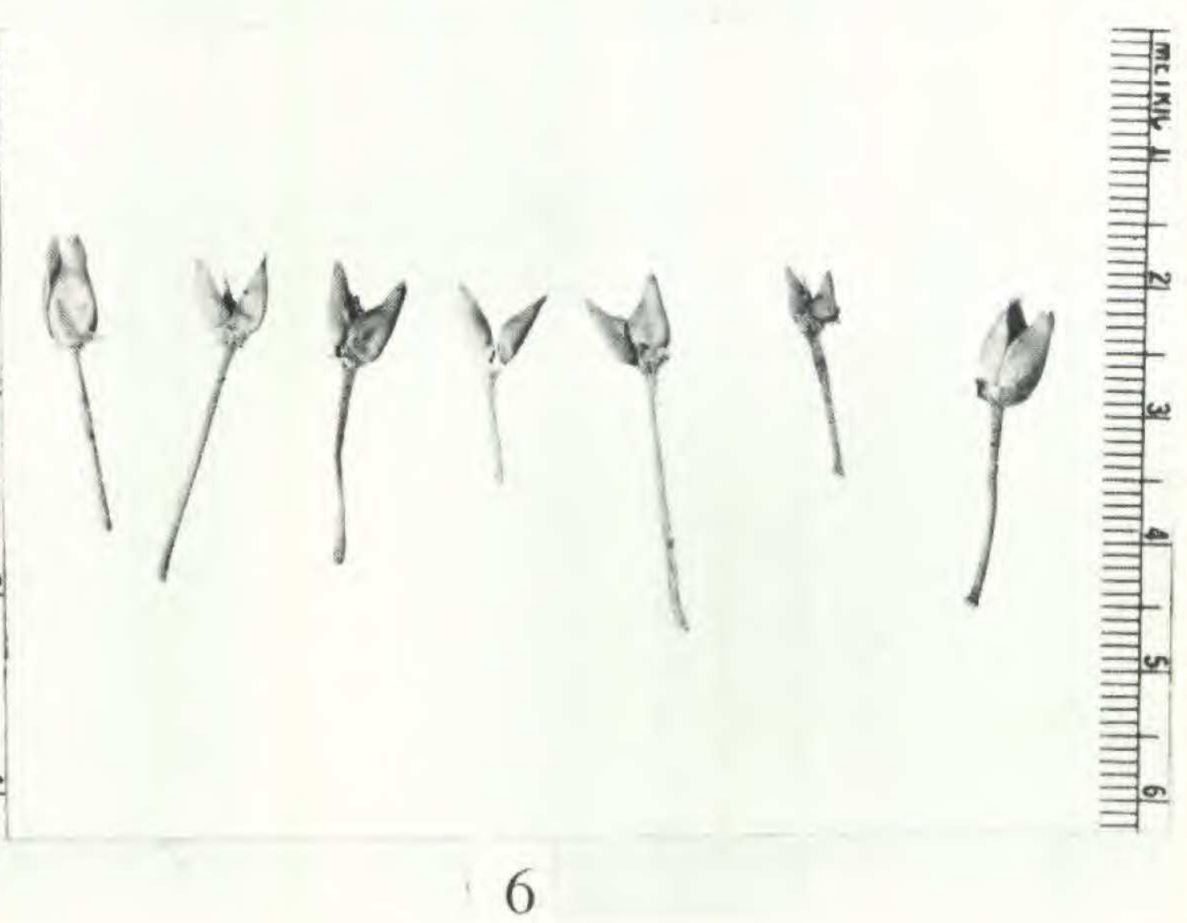
METRIC 1
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